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Semipermeable Membrane System for Subjecting Plants to Water Stress

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ABSTRACT

A system was evaluated for growing plants at reproducible levels of water stress. Beans (*Phaseolus vulgaris* L.) were grown in vermiculite, transferred to a semipermeable membrane system that encased the root-vermiculite mass, and then placed into nutrient solutions to which various amounts of polyethylene glycol (PEG) 20M were added to control solution water potential. The membrane (Spectrapor 1) had a minimum molecular weight cutoff that excluded the PEG 20M. The plants equilibrated with the nutrient solution within 1 to 4 days, and exhibited normal diurnal water relations. Use of the semipermeable membrane system to induce water stress reduces many of the problems associated with hydroponic media.

Numerous investigators have attempted to grow plants at specific levels of water stress by bathing the roots in a nutrient solution where the water potential is controlled by adding osmotic compounds such as PEG[1] (6–8). However, the presence of PEG in the nutrient solution may cause side effects because significant amounts of PEG are absorbed by the plants (6, 8). PEG reduced P uptake, translocation (3, 10, 11), and the O_2 content of nutrient solutions (9).

Zur (15) showed that it was not necessary to place a plant directly into a PEG solution to control its water potential. He (15) and subsequent investigators (2, 4, 16) encased soil-grown plants within semipermeable membranes which were immersed within nutrient solutions containing an osmotic agent (PEG). The membrane excluded the PEG from the roots but let water and nutrients diffuse to the roots. However, no attempts were made to determine how quickly the soil-plant-air continuum reached a steady-state; if the membrane system overcame the PEG effects on P uptake and translocation, or O_2 availability; or whether the membrane excluded PEG from the plant. The following work was done to explore these problems.

MATERIALS AND METHODS

Plant Growth. Beans (*Phaseolus vulgaris*, L. cv. Pinto 111) were grown in vermiculite[2] in plastic tubular containers (pine cells) with a volume of 66 cm³ (1) and watered daily with a modified Hoagland solution. Plants were grown on a 16-hr light 8-hr dark cycle in both the growth chamber and greenhouse. Light intensity in the growth chamber was 17,000 lux and in the greenhouse the sunlight was supplemented with artificial light to provide a minimum intensity of 15,000 lux. The day/night temperatures in the growth chamber were 30-23/23 C and 30/18 C for the greenhouse.

Water Stress System. Plants were grown in the pine cells approximately 21 days until the roots systems were well developed when they were transferred to modified pine cells in which 65% of the surface area (76 cm²) had been removed and enclosed with a water-rinsed semipermeable membrane. Dialysis tubing or seamless cellulose tubing (Spectrapor 1) with minimum mol wt exclusion limits of 12,000 to 14,000 and 6,000 to 8,000, respectively, were used. The membranes were cut over twice as long as the pine cells, twisted at the base, and the remainder brought back to the top of the pine cell and the plant-membrane systems were fitted through holes in the lids of polyethylene containers. The plant-membrane systems were equilibrated for 2 hr in distilled H_2O and transferred to nutrient solutions containing varying amounts of PEG 20M (average mol wt 14,000–16,000). In the solutions the membranes collapsed against the root-vermiculite mass providing good contact between membrane and root-vermiculite mass.

Chemical Measurements. Dissolved O_2 in the PEG solutions was measured with a membrane-covered polarographic probe and compared to measurements made with a Van Slyke manometer. PEG was extracted from plant tissue and quantified using Lawlor's method (8) except the PEG was extracted in 0.2 M borate buffer (pH 9) for quantitative recovery. For determining the elemental composition of the plants, leaves and stems were oven-dried and acid-digested (12). Organic N_2 was measured using the phenol-hypochlorite reaction (14) and P was determined using the phosphomolybdenum blue complex (13).

RESULTS

Equilibration Studies. When leaf water potential reaches a level less than the solution water potential, the plant should extract water from the solution and the solution water potential should control leaf water potential. The leaf water potential of plants without membranes and with membranes immersed in −1 bar PEG decreased from −3.5 to −6 and −3.5 to −5.2 bars, respectively, over the experimental period (Fig. 1). The leaf water potential of the −6 bar treatment decreased below the solution water potential 4 days after the treatment began and then varied between −6.6 and −8.2 bars for the remainder of the study. Vigorous, new root growth occurred between the vermiculite and the membranes in both the solution treatments, although there was less new root growth at −6 bars than at −1 bar.

When air temperature was varied between 23 and 30 C during equilibration, leaf water potential of plants at −1 bar varied approximately ± 0.5 bars over the 72-hr sampling period. In

[1] Abbreviation: PEG: polyethylene glycol.

[2] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Environmental Protection Agency, and does not imply its approval to the exclusion of other products that may also be suitable.

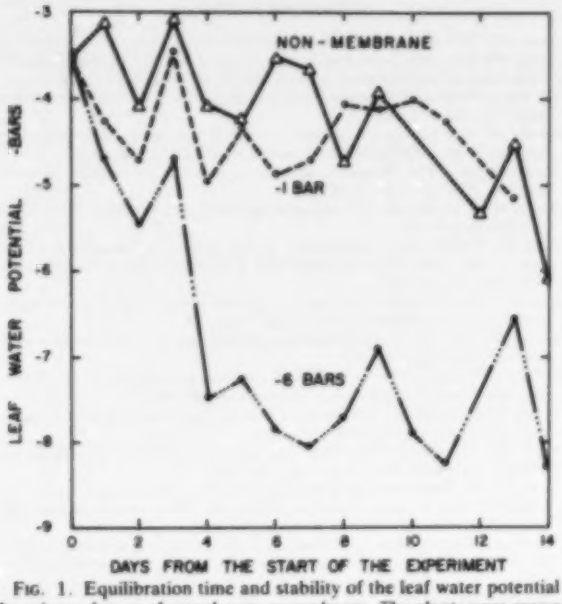

FIG. 1. Equilibration time and stability of the leaf water potential of the primary leaves of greenhouse-grown beans. The plants were grown in the greenhouse and placed in the different treatments at day 0. The plants in the nonmembrane treatment were left in their original pots and the others were transferred to the semipermeable membrane systems. Osmotic potentials of −1 and −6 bars refer to the solution outside the membranes. Leaf water potentials were estimated with a pressure bomb and taken daily between 1430 and 1630 PST. Each mean is the average of five observations and SE = 0.7 bars. The relative growth rates of the leaves were 0.05, 0.07, and 0.04 day^{-1} for the nonmembrane, −1 and −6 bars, respectively.

contrast, when plants were subjected to −6 bars, the leaf water potential decreased to 7.8 ± 0.5 bars within 24 hr and varied less than ± 1 bar for the remaining 48 hr.

To determine if plants in the semipermeable membranes exhibited diurnal changes in leaf water potential, plants were equilibrated for 6 days in solutions containing varying amounts of PEG and leaf water potentials were measured at various times during the next 24 hr. In the light, leaf water potentials decreased 35, 64, and 24%, respectively, in the −1, −5, and −10 bar treatments. After the lights went off, the leaf water potential recovered to its original level. The changes in leaf water potential were similar to anticipated normal diurnal changes.

PEG Effects on the Plant. Plants were grown in one to three layers of dialysis tubing, or a single thickness of Spectrapor membrane at −6 bars to determine the amount of PEG that passed through the membrane into the root-vermiculite mass (Table I). Increasing the thickness of dialysis membrane around the roots decreased PEG content. Each increase in membrane thickness decreased PEG uptake by about 50%. Less PEG entered the root-vermiculite mass with Spectrapor membrane than with dialysis membrane. When three thicknesses of dialysis membrane were used there was a significant decrease in the leaf water potential, suggesting that the membranes were retarding water flow into the plant.

To determine if the plants were using O_2 from the PEG solutions around the roots, the O_2 levels in solutions with (T) and without plants (C) were monitored daily for 7 days. On the 1st day of the experiment the O_2 levels ranged from 9 ± 1 mg/l at −1 bar to 6 ± 1 mg/l at −10 bars. The difference in the O_2 levels in the solutions (T−C) was calculated daily for each water stress level. If the plants used O_2 from the solution, the O_2 content would decrease. However, the slopes of the linear regression equation relating O_2 content and duration of the experiment were zero (−1 bar) and positive (−5 and −10 bars) indicating that the plants were not using O_2 from the nutrient solution.

The concentration of organic N_2 and total P in the plant tissue was measured to determine if the presence of PEG in the solution affected these components (Table II). There was no significant effect of the PEG-induced water stress on the N_2 concentration of the leaves, but the N_2 content of the stems was elevated above controls in the −6 bar treatment. The P concentration of the leaves and stems increased as the time in solution increased.

DISCUSSION

The semipermeable membrane system described in this study is conceptually similar to previously published reports (2, 4, 15, 16). The major differences were the use of a different membrane (Spectrapor 1) and of PEG 20M as the osmotic agent. In previous studies (2, 4), a membrane with a minimum mol wt exclusion of 12,000 to 14,000 (personal communications, Dr. J. Graham, Union Carbide Co.) and PEG 6,000 (mol wt of 6,000) were used. This combination of PEG and membrane exclusion size allowed a moderate amount of PEG to pass through the membrane into the root environment (8). Our data suggest that the use of a high mol wt PEG such as 20M and a membrane with a lower mol wt exclusion limit significantly reduced the amount of PEG that passed through the membrane.

In hydroponic solutions, plants absorbed up to 14% of the PEG in solution and transported it unmetabolized through the plant (6, 8). PEG contents of 1 to 3 mg/g fresh weight for roots (10), 0.5 to 2 mg/ml of plant sap (6) have been reported.

The PEG that diffused across the semipermeable membrane into the root-vermiculite mass could have resulted from low mol wt PEGs or from bacterial degradation of PEG (5). Bacterial

TABLE I. Influence of Various Membrane Types and Thicknesses on Leaf Water Potential and the PEG Concentration on the Root-Vermiculite Mass[1]

Membrane Combinations[2]	Leaf Water Potential Bars	PEG 20M Content[3] mg/g Fresh Wt.
Single Dialysis Membrane	-7.7	3.8
Two Dialysis Membranes	-8.4	2.0
Three Dialysis Membranes	-9.8	0.9
Spectrapor 1	-7.9	0.4

[1]The PEG was extracted from the root-vermiculite mass of plants grown in the membrane system for 7 days at -6 bars. Each mean is based on 7 observations. SE = 0.3 for both leaf water potential and PEG content.

[2]Minimum molecular weight exclusion limits for dialysis membrane and Spectrapor 1 are 12-14,000 and 6-8,000, respectively.

[3]Expressed as g Fresh Wt. of the root and vermiculite jointly.

TABLE II. Influence of PEG Induced Water Stress on the Concentration of N and P in Plant Tissue (mg/g)[1]

Chemical Constituent	Plant Component	Water Stress Level (Bars)	Days in PEG Solutions 0	4	7
Organic Nitrogen	Leaves	-1	43.9	42.9	42.3
		-6	41.9	43.5	42.3
	Stem	-1	16.7	17.4	17.3
		-6	22.1	21.2	29.3
Total Phosphorus	Leaves	-1	4.0	3.1	2.9
		-6	3.8	5.9	6.7
	Stem	-1	2.0	1.8	1.8
		-6	2.5	3.4	5.3

[1]The concentration is expressed in mg of constituent/g dry weight. Each mean is based on 7 observations. SE for organic nitrogen and total phosphorus is 1.1 and 0.5, respectively.

degradation was not rapid, if it occurred, since the solution water potential of −5 and −10 bar solutions decreased only 0.4 and 0.1 bar, respectively, over the 7-day time period. The membranes surrounding the root-vermiculite mass showed visual disintegration only after 2 to 3 weeks in solution, which is similar to the time periods previously reported (5, 15). The low levels of PEG found in the root-vermiculite mass indicated that the membrane functioned for at least 7 days.

When PEG is used in hydroponic solutions, the aeration of the viscous solutions can cause foaming (6, 8) and can seriously lower the O_2 content of the solution (9). We found no net plant consumption of O_2 from the PEG solution by the roots. The O_2 requirements of the roots in the membrane system were apparently met via O_2 diffusion through the vermiculite rather than from the solution.

Although PEG in nutrient solutions inhibits P uptake and translocation by plants (3, 10, 11), we found an increase in the P concentration in the leaves and stems with time in the PEG solution suggesting that P uptake and translocation were not inhibited.

The membrane (Spectrapor 1) had a minimum mol wt cutoff that excluded the PEG 20M. The plants equilibrated with the nutrient solution within 1 to 4 days, and exhibited normal diurnal water relations. Use of the semipermeable membrane system to induce water stress reduces many of the problems associated with hydroponic media.

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